(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 February 2005 (10.02.2005)

PCT

(10) International Publication Number
WO 2005/012495 A3

(51) International Patent Classification[7]: C12P 21/06, C12N 1/14, 1/16, 15/00

(21) International Application Number: PCT/US2004/024786

(22) International Filing Date: 2 August 2004 (02.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/491,923 1 August 2003 (01.08.2003) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by the SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH** [US/US]; Office of Technology Transfer 6011 Executive Boulevard, Suite 325, Rockville, MD 20852 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **NEVILLE, David, M.** [US/US]; 9624 Parkwood Drive, Bethesda, MD 20814 (US). **WOO, Jung-Hee** [KR/US]; 259 Congressional Lane, Apt. 214, Rockville, MD 20852 (US). **LIU, Yuan-Yi** [CN/US]; 8415 Buckhannon Drive, Potomac, MD 20854 (US).

(74) Agents: **SPRATT, Gwendolyn, D.** et al.; Needle & Rosenberg, P.C., Suite 1000, 999 Peachtree Street, Atlanta, GA 30309-3915 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
31 March 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR EXPRESSION AND PURIFICATION OF IMMUNOTOXINS

(57) Abstract: In one aspect the present invention relates to a method of expressing an immunotoxin in Pichia pastoris strain mutated to toxin resistance comprising a) growing the *Pichia pastoris* in a growth medium comprising an enzymatic digest of protein and yeast extract and maintaing a dissolved oxygen concentration at 40% and above; and b) performing methanol induction with a limited methanol feed of 0.5-0.75 ml/min/ 10L of initial volume during induction along with a continuous inusion of yeast extract at a temperature below 17.5 °C., antifoaming agent supplied up to 0.07%, agitation reduced to 400 RPM, and the induction phase extended out to 163 h. In another aspect, the present invention relates to a method of purifying a nonglycosylated immunotoxin comprising a) loading a solution containing the nonglycosylated immunotoxin onto a hydrophobic interaction column; b) obtaining a first non-glycosylated immunotoxin containing eluant from the hydrophobic interaction column; c) loading the non-glycosylated immunotoxin containing eluant from step (b) onto an anion exchange column; d) obtaining a second non-glycosylated immunotoxin containing eluant from the anion exchange column by eluting the non-glycosylated immunotoxin with a sodium borate solution; e) diluting the concentration of sodium borate in the second non-glycosylated immunotoxin containing eluant from step (d) to about 50 mM or less; f) concentrating the diluted non-glycosylated immunotoxin containing eluant from step (e) over an anion exchange column; and g) obtaining a purified non-glycosylated immunotoxin from the anion exchange column.

WO 2005/012495 A3